THE INFLUENCE ON THE MORPHOLOGY AND STAINING OF B. DIPHTHERIÆ BY GROWTH IN MIXED CULTURES WITH STAPHYLOCOCCI AND STREPTOCOCCI*

W. D. STOVALL, EMERALD SCHEID AND M. STARR NICHOLS

SINCE Loeffler described the diphtheria bacillus there have been described from time to time various methods for differentiating the toxin from the nontoxin producers, the virulent from the avirulent bacilli. This has led to the production of various classifications based on morphology and staining characteristics. Corbett[1] worked out a classification for B. diphtheriæ, which combined the size and shape of the organism with its staining characteristic. Westbrook[2] and McDaniel prepared a more elaborate classification dividing the four main groups into subgroups. Their classification is based upon the size and shape of the organism together with its staining reaction, and they also attribute to certain forms virulent characteristics while certain others are avirulent, or less virulent. The solid types are avirulent types as a rule.

Because in the studies and classifications granules have been taken to indicate a virulent organism, many staining methods have been employed to bring out this characteristic of diphtheria bacilli. Loeffler's alkaline methylene blue has been used largely for this purpose. Corbett[1] employed a combination of this stain and acetic acid, and Coles[3] used Neisser's blue, Gram's iodine and Bismarck brown. Within the last few years Albert[4] and others have described special staining methods designed to assist in differentiating avirulent from virulent diphtheria bacilli.

The various classifications have left in the mind of many, false notions concerning virulence and morphology and staining characteristics, and every little while there appears some modification of the above mentioned staining methods for bringing out polar granules, and thereby distinguishing virulent from avirulent bacilli.

It was early shown by Reichenbach[4] that some solidly staining organisms are fully virulent, and many years ago Park stated that neither Neisser's stain nor any modification of it gave any information as to the virulence of the bacilli.

In our routine work we have to examine many nose and throat cultures for the diagnosis of diphtheria. We have been unable to derive any help in this work from any of the classifications or special stains. As we have observed it, there are so many varying conditions which influence the growth and morphological characteristics of these organisms that no regular system of diagnosis can be used. The thing which has interested us most is the influence on the morphology and staining characteristics of growth in mixed cultures. Throat cultures always contain a variety of organisms, and it is to be expected that the other organisms present influence the growth, and modify other characteristics of diphtheria bacilli in the culture. Our attention was called to this with cultures which we isolated in pure culture for virulence tests. It frequently happens that an organism may appear to be a solid staining organism in the mixed culture as grown from the throat and in pure culture turns out to be a typical beaded or banded type, or if there is not a change from a solid to a granular type there is a change in the type of some kinds so that it is not classified in the pure culture as it is in the mixed.

Because of this difference in the morphology and staining characteristics of diphtheria bacilli in mixed and pure cultures we determined to find out, if possible, what organisms in the cultures affect this change, and how our opinion as to the virulence or nonvirulence of diph-

* From the State Laboratory of Hygiene, Madison, Wisconsin.

theria bacilli in the cultures is affected by growth with these organisms. We accordingly selected two of the organisms which most frequently appear in the throat cultures which come to us, as the two organisms to grow with pure cultures of B. diphtheriæ. We secured a pure culture of a staphylococcus aureus and a streptococcus which formed rather long chains, the cocci occurring close in the chain, but which was not hemolytic. Pure cultures of B. diphtheriæ were secured from twenty throat cultures submitted for diagnosis. We used Westbrook's classification to classify these organisms. The diphtheria bacilli were classified in the throat cultures, in pure cultures, and when grown with staphylococci and streptococci. All these organisms were grown for twenty-four hours on ox blood serum medium made according to the Loeffler formula and washed down with salt solution. We were careful to see that the salt solution suspension of the organisms had the same density, that the suspensions were uniform in strength.

When we began the work we mixed equal quantities of suspensions of staphylococci and diphtheria bacilli, and equal quantities of suspensions of streptococci and diphtheria bacilli. These mixtures were shaken to insure mixing and a loopful from the mixtures was planted on tubes of Loeffler's blood serum. These cultures were allowed to grow twenty-four hours. From these growths stains were made with borax methylene blue, and the diphtheria bacilli classified according to morphology and staining. The culture was considered one of B. diphtheriæ of a given type depending upon the type which predominated. We also stained many preparations with Loeffler's alkaline methylene blue, but found no difference in the two stains, and discontinued the latter stain.

After the first trial it was apparent that we could not use equal amounts of the staphylococcus suspension and suspension of B. diphtheriæ because the staphylococci in that amount either overgrew or in some way inhibited the growth of the diphtheria bacilli so that none could be found for classification. We then mixed one-tenth of a cubic centimeter of the staphylococcus suspension with one cubic centimeter of the diphtheria bacillus suspension, and grew plantings of these as described above. It was not necessary to change the proportion of the streptococcus suspension to the diphtheria bacillus suspension so that we continued to use these two suspensions in equal amounts.

Virulence tests were done with all of the cultures of B. diphtheriæ. The reaction to galactose, dextrin and saccharose was recorded for each organism but is not given because we failed to stain from the tubes to see if we had a growth in every case. Because of this and some other omissions we feel that the results are not good.

The result of the classification of the bacilli according to Westbrook's classification is given in the accompanying table. It will be seen that of the twenty-one cultures studied in the original throat cultures, four (4) were determined as type A, five (5) as D or D2, ten (10) as B. Of the cultures which fell in the A type in the throat cultures all but one remained in that class when isolated in pure culture. This indicates that this organism was almost a pure culture as it was received for diagnosis. Throughout the study we observed this to hold true; that when an organism remained in the same type when isolated in pure culture as it was classified in the throat culture that the throat culture was almost a pure culture of B. diphtheriæ. As shown in the table, these organisms, type A, when mixed in the proportions described above with staphylococci changed from type A to type C, and when mixed with streptococci as described above they remain in the same type as in pure cultures. Thus it is seen that when mixed with staphylococci the organisms are reduced in size, become thinner and sometimes apparently longer, and stain as

heavily banded organisms while in pure culture they stain as large club shaped organisms with large granules. In many cases bands were so heavy and close together that it was easy to mistake these organisms for solid staining bacilli. This was a constant result of the growth of B. diptheriæ with staphylococcus, and if the Westbrook classification was too strictly followed resulted in placing the organism on morphological grounds outside of the group of diphtheria bacilli.

The effect or rather the absence of any effect of streptococci on the morphology and staining of B. diphtheriæ as shown in the table was a constant result. A study of the table reveals that all of the type A organisms in pure culture were also type A when grown with streptococci. This is also true for other types represented in the table.

The most striking effect of the staphylococcus upon the diphtheria organisms is shown in the table in the group D and D2 organisms. It will be noticed that in that group the D2 which proved to be nonvirulent was unchanged in morphology. It remained the same in all cultures. The other D2 which was so classified in the throat culture, in pure culture proved to be a type B organism, mixed with staphylococcus reverted to a D2 organism, that is B. Hoffmanni type, and mixed with streptococci was classified as type B, the same as in pure culture. This organism proved to be a fully virulent organism.

During the course of this work we frequently ran across an organism which in the mixed throat cultures had the appearance of a fully virulent type B organism but when isolated in pure culture stains showed a long thin granular organism which, although it showed no branching, looked as if it might be a branching organism. This organism was

MORPHOLOGY AND VIRULENCE OF B. DIPHTHERIAE

Throat Culture	Pure Culture	Mixed Culture Staphylococcus	Mixed Culture Streptococcus	Virulence	Reaction on Sugars		
					Galactose	Dextrin	Saccharose
A	B	C 1	B	+	+	+	—
A 1	A	C 1	A	+	+	+	—
A	A	C 1	A	+	0	0	0
A	A	C 1	A	+	+	+	—
D	C	C	C	+	+	+	—
D 2	B	D	B	+	+	+	—
D 2	D 2	D 2	D 2	—	—	—	—
DD 2	B	B	B	+	+	+	+
B	X	B	B2X	—	+	+	+
B	X	B	B	—	+	+	+
B	X	B	X	—	+	+	+
B	X	B	X	—	—	+	+
B	AB	BC	A	+	+	+	—
B	BA	CB	X	—	+	+	+
B	BA	D 1 C 1	B	+	0	0	0
B	B	D 1 C 1	BA	+	+	+	—
B	B	C	B	+	+	+	—
B	B	C	B	+	+	+	—
C	B	B	B	+	+	+	—
C	B	C 1	B	+	+	—	—
C	B	C 1	B	+	+	+	—

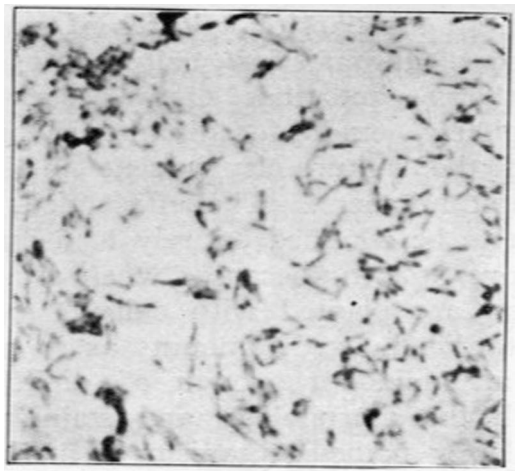

PLATE I

FIGURE 1—Typical Beaded Type A Culture of B. Diphtheriæ Grown in Pure Culture.

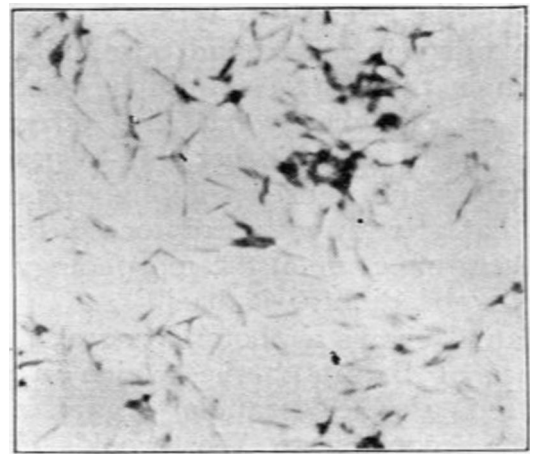

PLATE II

FIGURE 1—Type X Organism Grown in Pure Culture.

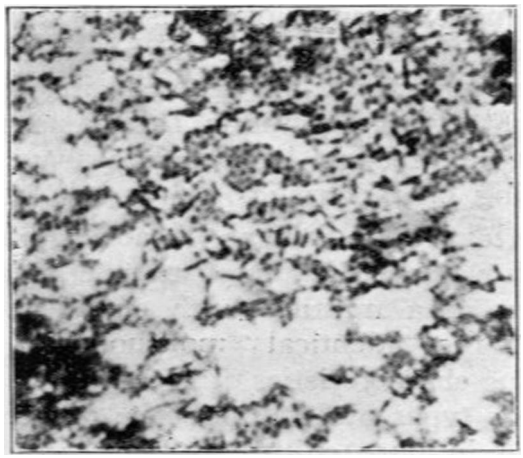

PLATE I

FIGURE 2—Same Culture as the One Shown in Figure 1 but Grown with Staphylococci.

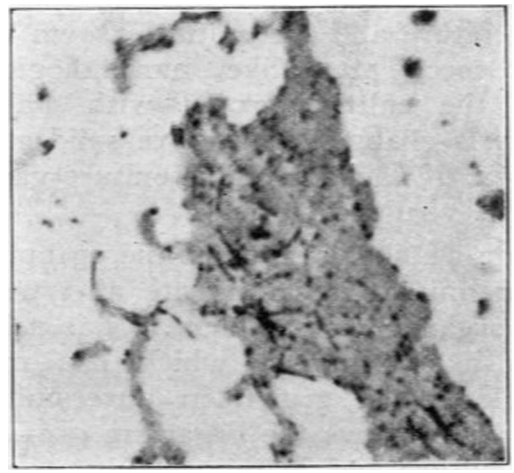

PLATE II

FIGURE 2—Type X Organism Grown with Staphylococcus.

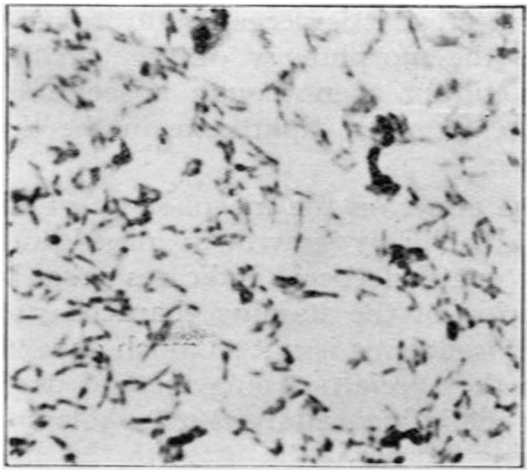

PLATE I

FIGURE 3—Same Culture as the One Shown in Figures 1 and 2 but Grown with Streptococci.

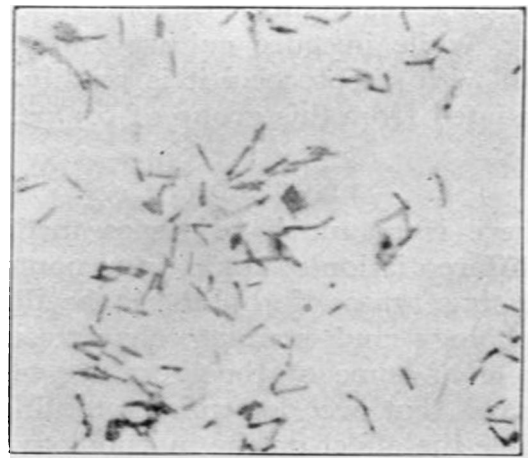

PLATE II

FIGURE 3—Type X Organism Grown with Streptococci.

always negative to virulence tests. We called this organism type X and its morphology in mixed and pure culture is shown in the plate. It will be observed here as elsewhere that the effect of growth with staphylococcus is to produce a smaller and denser staining organism, and that growth with streptococci has no apparent effect upon the morphology and staining of diphtheria bacilli.

Plate I shows a photomicrograph of a culture of B. diphtheriæ, which in pure culture is a typical granular organism and which, according to Westbrook's classification, showed the predomination of a type A organism. This plate shows this organism grown in pure culture and mixed cultures both with staphylococci and streptococci. It is evident that the pure culture and the culture grown with streptococci are alike morphologically while the culture grown with staphylococci is changed to a more solid staining type of bacillus. This culture gave a positive virulence test.

Plate II shows a photomicrograph of the organisms we designate as type X. This organism is avirulent. It is a long thread-like organism which has many granules when grown in pure culture. This plate shows the organism grown in pure culture and in mixed cultures with staphylococci and streptococci. It is apparent that when grown with staphylococci this organism is reduced in size and stains very much more solid and then can not be distinguished from B. diphtheriæ, while in pure culture and when grown with streptococci it is readily differentiated from this group of organisms.

DISCUSSION

There is so much reference made to the differentiation of toxin from nontoxin producing types of diphtheria bacilli by morphology and staining characteristics in spite of some of the literature to the contrary that we determined to find if possible the effect on the morphology and the staining characteristic of B. diphtheriæ of growth in mixed cultures. Our work indicates that there is a definite effect on the part of cultures of staphylococcus aureus for cultures of B. diphtheriæ, and that staphylococci will completely eradicate diphtheria bacilli from the culture if present in large enough numbers. The effect does not seem to be confined entirely to a matter of overgrowth because when staphylococci are planted with cultures of B. diphtheriæ in such amounts as to allow for an abundant growth of diphtheria bacilli the morphology and staining of the latter named organism is markedly changed. This change is so marked as to completely change the classification of the diphtheria bacilli when Westbrook's classification is used. This influence is so constant and so marked in some cases as to make it impossible to use Westbrook classification for routine diagnostic work.

Streptococci when grown in equal amounts with diphtheria bacilli seem to effect no change in its size, shape or staining characteristics.

Certain nonvirulent beaded bacilli which we have designated as type X when grown in a mixture with staphylococci have the identical morphology and staining characteristics of type B of Westbrook's types of B. diphtheriæ, but when grown in pure culture is readily differentiated from B. diphtheriæ.

The result of this work indicates that when diphtheria-like bacilli are found in throat cultures in which staphylococci are growing that it is necessary to isolate and study the morphology of the bacillus in pure culture even though it seems quite remote from B. diphtheriæ in the throat culture.

The effect on the morphology of diphtheria bacilli by growth in mixed culture with staphylococci indicates that the variation of the staining characteristic of these organisms is not so much dependent upon the special staining method used as upon the influence on the morphology and staining reaction by growth in mixed culture. It appears to us that this change in morphology and staining re-

action is due to some change in the composition and structure of the organisms; that some change in the physiology of the organisms has been brought about. If we are correct in this supposition then it is easy to explain why stains can not be relied upon to differentiate toxin from nontoxin producers. If changes have been brought about which interfere with granule formations then, of course, no stain will be able to stain granules. It is certain that in mixed cultures with staphylococci diphtheria bacilli will stain as solid bacilli while in pure culture they are distinctly beaded.

Our observations of the influence of staphylococci on the staining of avirulent beaded bacilli indicate that in this case the beading may be more pronounced; that is, the bacilli stain more solid, producing an effect which makes the organism more closely resemble what is usually accepted as good morphology for true diphtheria bacilli. The result is that even when the stain does bring out granules it can not be depended upon to differentiate toxin from nontoxin producers because of the influence of growth with staphylococci on the staining reaction of the bacilli.

REFERENCES

1. The Result of 950 Bacteriological Examinations for Diphtheria Bacilli During an Outbreak of Diphtheria at Cambridge and Chesterton. Journal of Hygiene, IV, 1901.
2. A Preliminary Communication Bacillus Diphtheria and Its Variants in a School in which Diphtheria Was Epidemic. British Medical Journal, IV, 1898.
3. A Modification of Neisser's Diagnostic Stain for the Diphtheria Bacillus. British Medical Journal, V, 1899.
4. A Classification of Diphtheria Bacilli Based on the Toluidin Blue-Iodine Method of Staining. American Journal of Public Health, December, 1920.

REPORT OF THE COMMITTEE OF THE STATE AND PROVINCIAL HEALTH AUTHORITIES ON RELATION OF MEDICAL MEN AND HEALTH OFFICIALS

W. S. RANKIN, M.D., FELLOW A. P. H. A.
Secretary North Carolina State Board of Health, Raleigh, N. C.

Read before the Conference of State and Provincial Health Authorities at Washington, May 14-15, 1923.

IN CONSIDERING the relation of medical men and health officials the following questions are encountered:

1. What is the field of medicine?
2. What is the field of public health?
3. Do these fields merge?
4. If merged what understanding and relation should be established between medical men and health officials with public welfare the controlling motive?

WHAT IS THE FIELD OF MEDICINE?

Medicine as it is practiced to-day by the rank and file of the medical profession concerns itself more especially with the diagnosis and treatment of diseases that have advanced to a stage where they incapacitate the afflicted and interfere with productive efficiency or the enjoyment of life. Most of these diseases occupying the time and thought of the medical profession have reached what Sir James MacKenzie classifies as the "advanced stage" and many have approached the "final stage"; few diseases, relatively speaking, are treated by the profession in the "early stage," and fewer still in the "predisposing stage."

Nevertheless, it is true that the medical profession to-day is giving more treatment for diseases that are in the early stage and to patients who are predisposed than ever before. There is a strong, irresistible, unceasing current in medicine moving from the obviously pathological toward the more physiological conditions of life. This tendency of medicine to find its patient before irreparable damage has been done and to treat disease in its more curable stages has been made possible (1) by a larger appreciation on the part of both physician and patient of early